

## **Abstract:**

Title: HPLC determination of delta-9-tetrahydrocannabinol

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The aim of my work was to develop a suitable analytical method for forensic application (without heat treatment of examined sample during analysis to prevent an increase of  $\Delta^9$ -THC content in the sample) to detect delta-9-tetrahydrocannabinol from cannabis. I optimised and validated conditions of high-performance liquid chromatography (HPLC) method for the quantitative analysis of delta-9-tetrahydrocannabinol ( $\Delta^9$ -THC) in the n-hexane extract of marihuana. Quantitation was accomplished with the internal standard (IS) method using beta-17-estadiol acetate. The separation was achieved on a reverse-phase C18 column, using methanol and water (90:10) as mobile phase. The compounds were eluted isocratically at a flow rate of 1ml/min. The compounds were analyzed with fluorescence detection at 220nm/317nm. The retention time of  $\Delta^9$ -THC and the IS was 9.97min and 5.25 min, respectively, and the total run time of the assay was around 10 min. The validation characteristics included linearity, accuracy, precision, limit of detection and quantification and robustness. The calibration curve was linear over the range of 0.56-35.71  $\mu\text{g/ml}$  with a correlation coefficient of  $r > 0.9998$ . Limit of detection (LOD,  $S/N = 3$ ) and limit of quantification (LOQ,  $S/N = 10$ ) values of the  $\Delta^9$ -THC were 0.55 and 1.84  $\mu\text{g/ml}$ , respectively. The relative standard deviation (RSD) value of the repeatability was reported within 2.2–10.8 %. The average recovery of  $\Delta^9$ -THC was 105 %. Validation acceptance criteria were in all cases in accord with Guidance for industry- Bioanalytical Method Validation.

